Appendix

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Appendix Figure Legends

Appendix Figure S1. Microglial *Gpr56* transcript expression across different developmental stages. Data was obtained from the published RNAseq data (Matcovitch-Natan et al., 2016).

Appendix Figure S2. Exposure of PS on Ba/F3 cells after A23187 treatment.
(A) Before treatment, Ba/F3 cells were barely detected by Annexin V, which binds to PS. (B) With treatment of calcium ionophore A23187, the majority of Ba/F3 became Annexin V positive, indicating Ba/F3 cells externalized PS to the outer leaflet of plasma membrane.

Appendix Figure S3. QPCR.
(A) A standard curve for the qPCR2 reaction was performed using 1:2 serial dilution of microglial cDNA. (B) A standard curve for the qPCR1 reaction was performed.

Appendix Figure S4. Quantification of vGlut2 presynapse and Homer1 postsynapse densities.
(A) Increase vGlut2^{+} presynapse densities were found in *Gpr56 null* mice, not in *Gpr56 S4* mice. (B) No significant change of Homer1 postsynapse density was shown in *Gpr56 null* and *S4* mice. N=10 (Ctrl), N=6 (*Gpr56 null*), N=3 (*Gpr56 S4*). *$p < 0.05$, **** $p < 0.0001$, one-way ANOVA with Tukey's post-hoc test.

Appendix Figure S5. Restricted GFP expression in microglia driven by *Cx3cr1-Cre*.

(A) A scheme showing the generation of *Rosa-GFP*^{fl} transgenic mice and *Rosa-GFP*$^{fl/+}$; *Cx3cr1-Cre*$^{+/-}$ mice. (B) GFP was barely observed in *Rosa-GFP*$^{fl/+}$ mice. (C) GFP expression driven by *Cx3cr1-cre* was restricted in microglia in the dLGN and cortex. Scale bar, 100 μm.

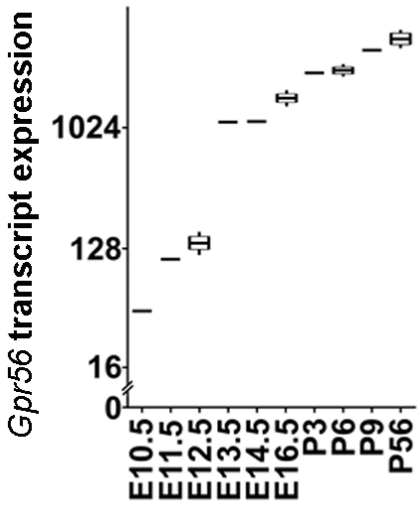
Appendix Figure S6. Quantification of vGlut2 presynapse and Homer1 postsynapse densities in early dLGN development.
(A) Increase vGlut2^{+} presynapse densities were found in CKO mice at P5 and P10. (B) No significant change of Homer1 postsynapse density was seen in CKO mice. N = 3 (P0), N = 4 (P5), N=3 (P10). *$p < 0.05$, ** $p < 0.01$, two-way ANOVA followed by Bonferroni's post hoc test.

Appendix Figure S7. Retinogeniculate synapses in CKO and controls using SIM.
Top panels show original images taken by SIM. Middle panels show 3D rendered images after processing in Imaris. Bottom panels show Homer1 spots (magenta) that are within the distance of 300nm from vGlut2 surface (green). Scale bar, 5 μm.

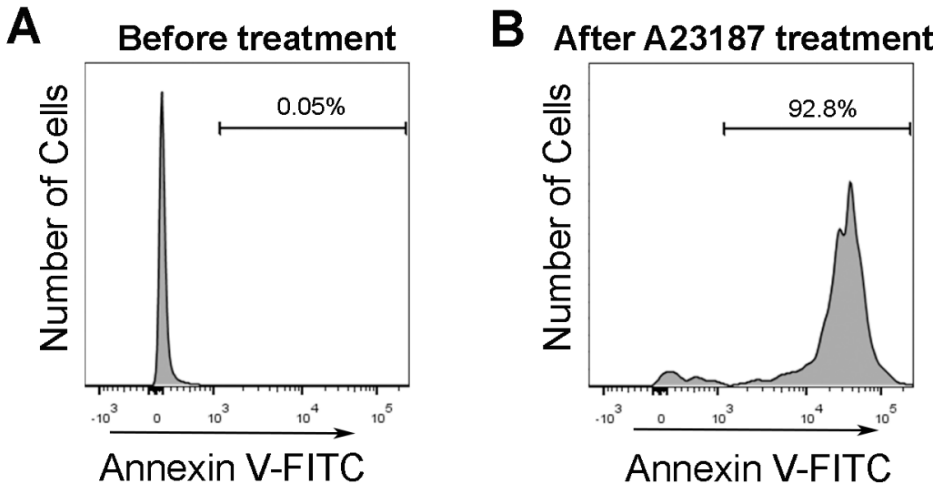
Appendix Figure S8. The variance of R-value varies from cranial to caudal LGN.
(A) Images of superior colliculus after 24 hours anterograde labeling of RGC by CTB488 and CTB647 at P30, indicating efficient RGC labeling by CTB. (B) Variance of R-values varies from caudal LGN to cranial LGN.

Appendix Figure S9. *Gpr56* CKO mice show no significant difference in paired pulse depression. Paired pulse depression was recorded on dLGN slice from P28-P34 mice. Given that optic inputs usually demonstrate paired pulse depression, and cortical inputs show paired pulse facilitation, this data indicates that the optic tracts and not cortical inputs were stimulated. N = 14 (Ctrl), 23 (CKO) cells from 5, 7 mice. P = 0.694 by Student's t-test.

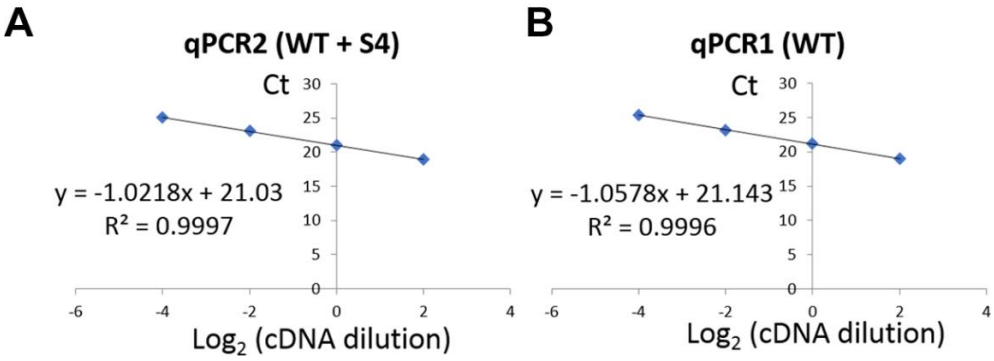
Appendix Figure S1



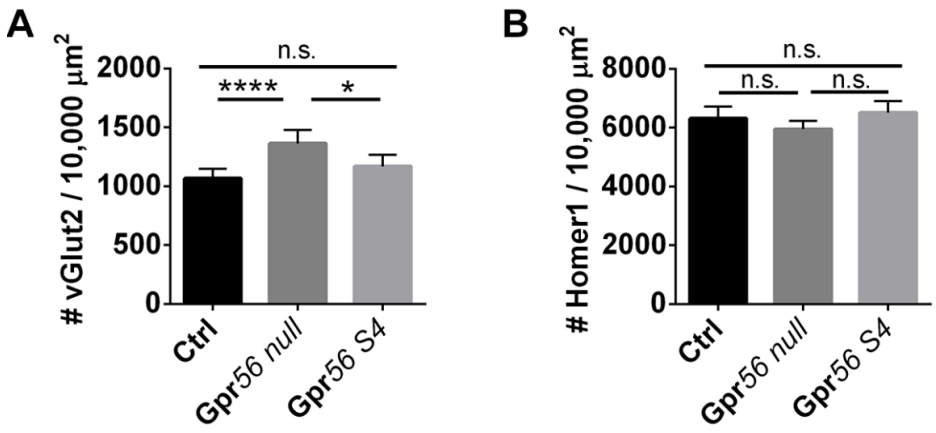
Appendix Figure S2



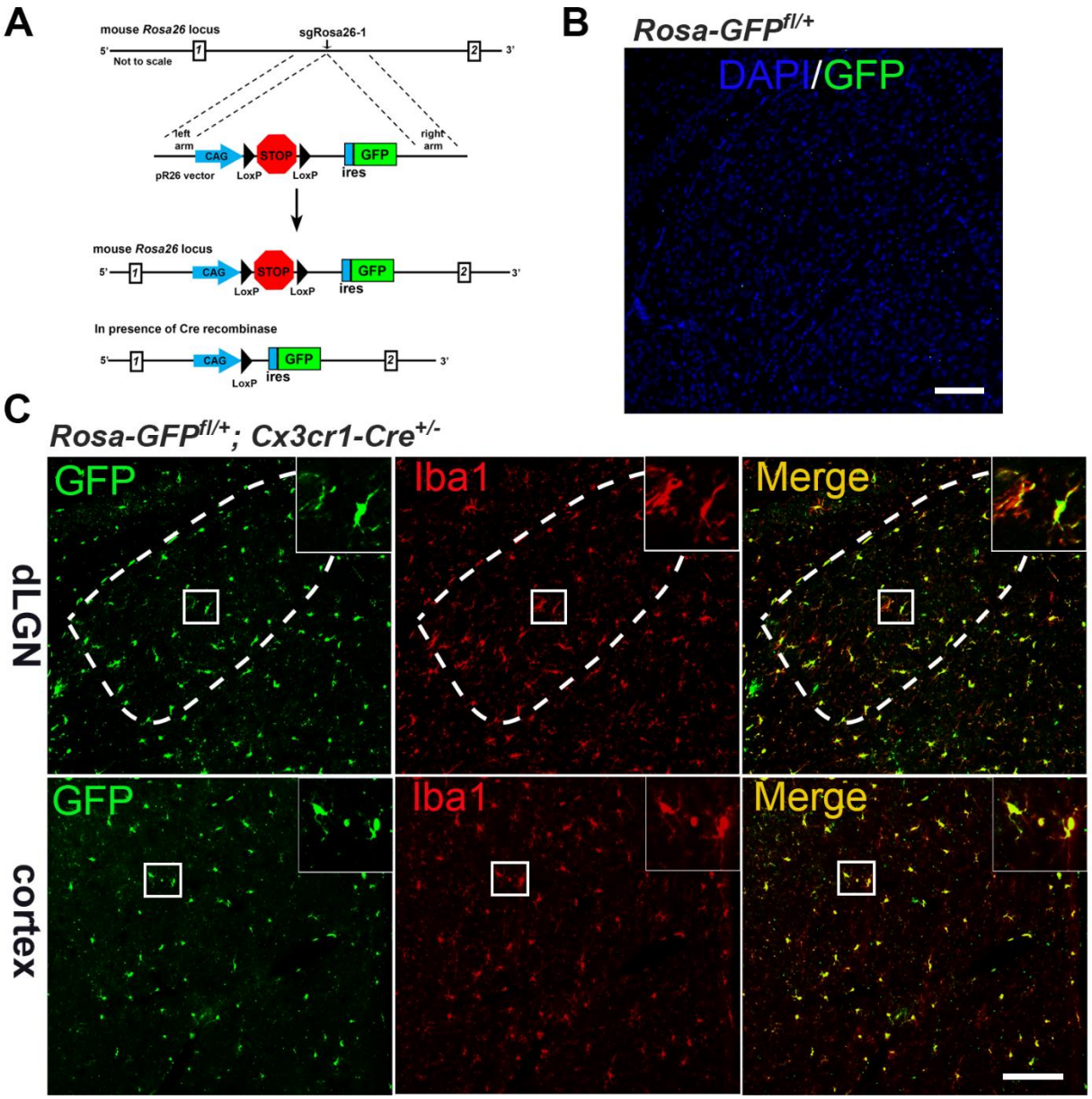
Appendix Figure S3



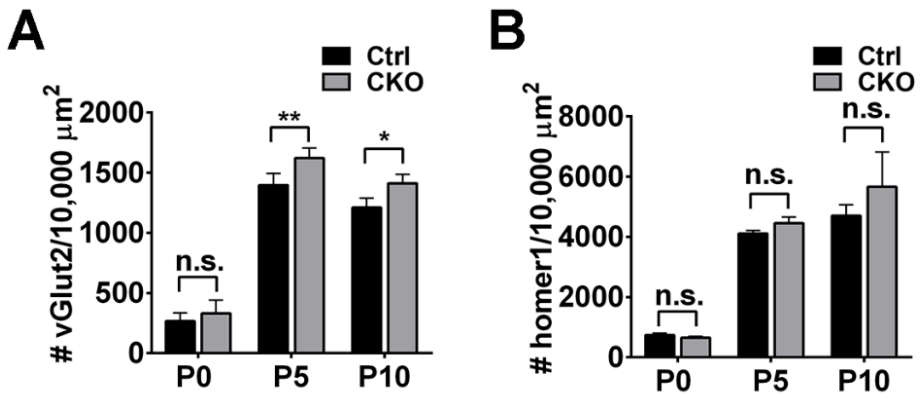
Appendix Figure S4



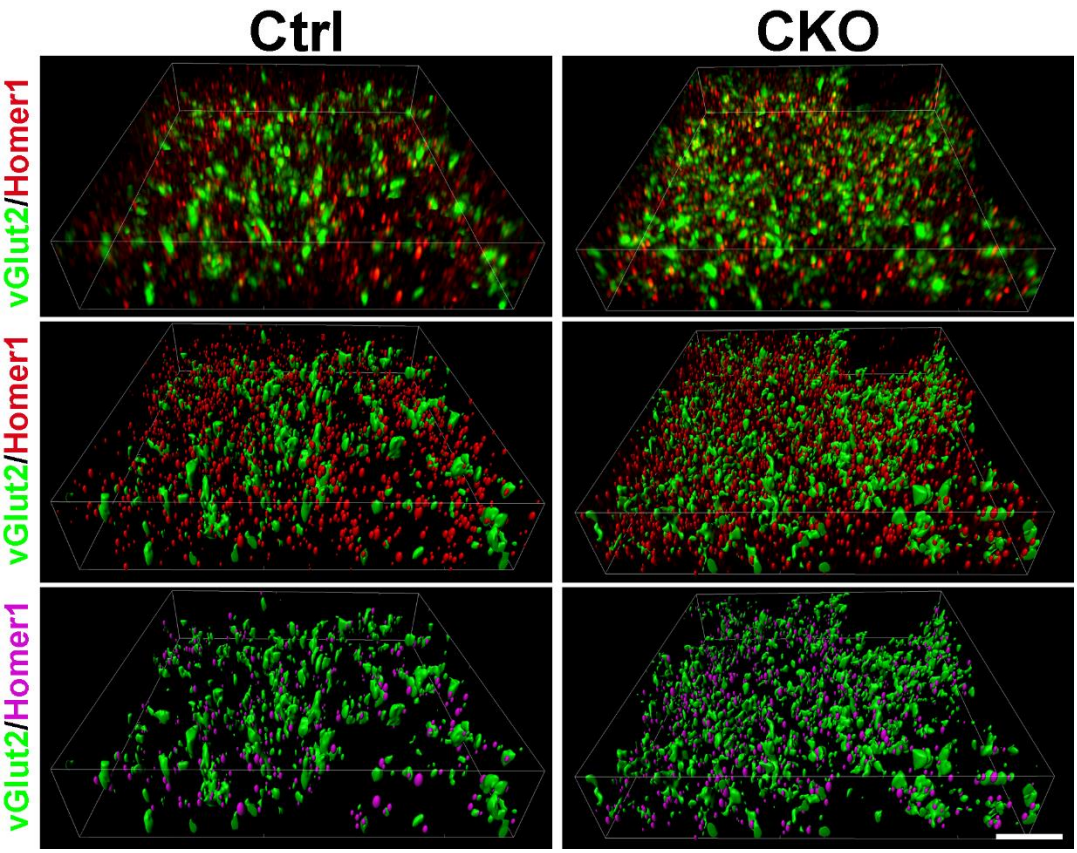
Appendix Figure S5



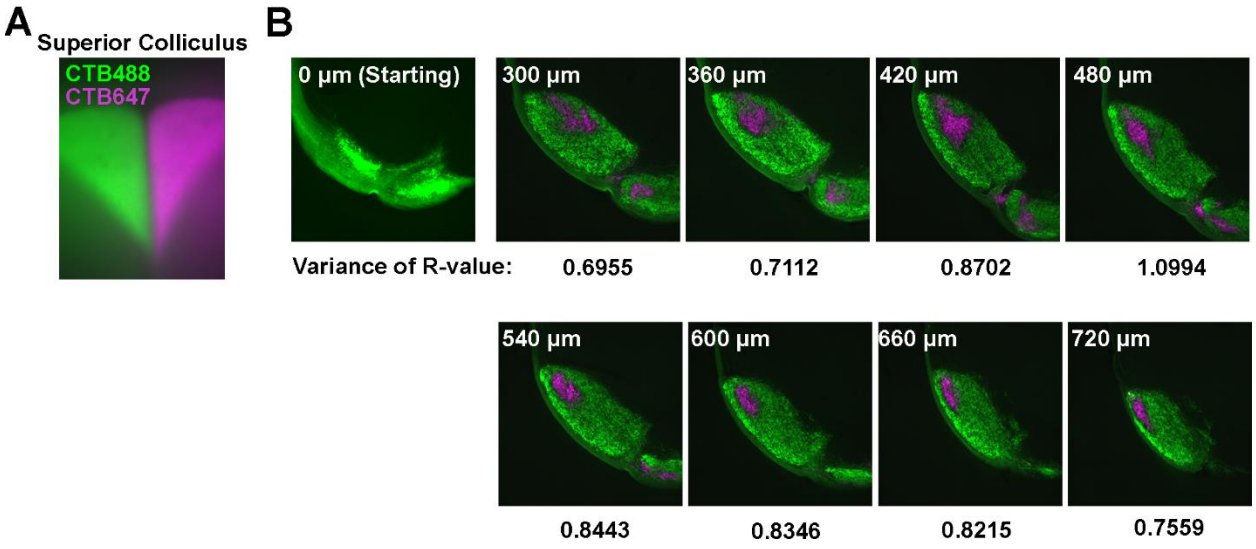
Appendix Figure S6



Appendix Figure S7



Appendix Figure S8



Appendix Figure S9